Atypical CD4+/CD8+ Lymphocytosis and Prolonged Pancytopenia Associated with Human Herpesvirus 6 Reactivation after Autologous Peripheral Blood Stem Cell Transplantation

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CONFLICTS OF INTEREST

The authors of this manuscript have no affiliations with or involvement in any organization or entity with any financial interests or non-financial interests in the subject matter or materials discussed in this manuscript.

MICROABSTRACT

Reactivation of latent human herpesvirus-6 (HHV6) can be associated with prolonged pancytopenia following allogeneic hematopoietic cell transplantation (HCT). Reports of HHV6 complications in autologous HCT is limited. This case report describes HHV6 reactivation complicated by atypical lymphocytosis of characteristic CD4+/CD8+ cells and subsequent prolonged pancytopenia after autologous peripheral blood stem cell transplantation (PBSCT) for multiple myeloma (MM).

ABSTRACT

Introduction:

Reactivation of latent human herpesvirus 6 (HHV-6) often occurs after hematopoietic cell transplantation (HCT). Following autologous HCT, HHV-6 reactivation has been associated with a slight delay in hematopoietic recovery, however, it has not been associated acute alterations in peripheral leukocytes.

Case Report:

In this report, we describe prolonged pancytopenia as a consequence of HHV-6 reactivation in a 63-year-old man who underwent autologous peripheral blood stem cell transplantation (PBSCT) for multiple myeloma. Preceding this pancytopenia, there was a transient atypical lymphocytosis consisting of double positive CD4+/CD8+ T-lymphocytes.

Results:

Flow cytometry of peripheral blood on day +11 showed atypical lymphocytes positive for CD4 and CD8. Quantitative PCR was positive for HHV6 with 250 copies/mL and negative for CMV and EBV. Bone marrow aspirate and biopsy on day +31 showed marked erythroid hyperplasia

(M:E ratio 0.18) and a small population (1.7%) of T-cells coexpressing CD4 and CD8. On day +38, the ANC was 0.99 x 10^6/L.

Conclusion:

This instructive case demonstrates that HHV6 reactivation after autologous PBSCT can be associated with atypical lymphocytosis of characteristic CD4+/CD8+ cells and subsequent prolonged pancytopenia. HHV6 is known to induce expression of CD4 in CD8+ lymphocytes, and that viral reactivation likely led to the observed CD4+/CD8+ lymphocytosis. As autologous HCT remains a component of MM comprehensive therapy, transplant infectious diseases specialists and HCT physicians are encouraged to have a low index of suspicion for HHV6 reactivation when assessing MM patients who develop persistent fevers, rashes, atypical lymphocytosis or prolonged marrow hypoplasia after autologous HCT.

Keywords: human herpesvirus 6, CD4+/CD8+ lymphocytes, autologous peripheral blood stem cell transplantation

INTRODUCTION

Reactivation of latent human herpesvirus 6 (HHV-6) is common after allogeneic hematopoietic cell transplantation (HCT; transplantation of bone marrow, peripheral blood stem cells, or cord blood), occurring in approximately 40-70% of those recipients[1,2]. After autologous HSCT, the reported incidence of HHV-6 reactivation is either similar to[1,2,3] or lower than (8-19%) that observed after allogeneic HCT[4,5,6,7]. Much of the literature agrees there is no statistically significant difference between HHV-6 reactivation in autologous and allogeneic recipients[1,2,5,8,9]. Manifestations of HHV-6 reactivation following either autologous or allogeneic HCT may include fever and rash[2,10]; after autologous HCT, diarrhea and pneumonitis may also occur[4]. Reactivation of this virus often occurs during pancytopenia and thus is not associated with acute elevations or alterations in peripheral blood leukocytes, potentially informative diagnostic findings that can be observed with HHV-6 infection in normal individuals[11,12].

Previous literature reports at most a modest delay in hematological recovery following active HHV-6 infection for both allogeneic and autologous HCT, with a median duration of neutropenia of 12.5 days (range, 6-21 days)[2]. However, this does not drastically differ from neutrophil aplasia experienced by patients undergoing their first autologous PBSCT, where the median time to neutrophil engraftment was 12 days (range, 9-25 days)[13]. Extremely prolonged pancytopenia and graft failure associated with HHV-6 reactivation are well-recognized after allogeneic HCT[14,15], but to our knowledge have not been extensively reported after autologous HCT.

In this report, we describe a transient atypical lymphocytosis with CD4+/CD8+ cells followed by prolonged bone marrow hypoplasia as manifestations of HHV-6 reactivation in a patient who underwent autologous peripheral blood stem cell transplantation (PBSCT) for

multiple myeloma. The CD4+/CD8+ immunophenotypic profile is historically associated with intrathymic T cell development. However, circulating double positive T-lymphocytes can be found in healthy individuals and has been linked to an adaptive immune response to various pathogens[16]. Additionally, previous literature has linked HHV-6 to the induction of CD4 expression in CD8+ lymphocytes[17].

CASE REPORT

A 63-year-old man was diagnosed with IgA kappa multiple myeloma (Durie-Salmon Stage IIIA and ISS stage 3). He received initial treatment with lenalidomide, bortezomib, and dexamethasone; pomalidomide replaced lenalidomide for subsequent cycles because of intolerance to lenalidomide. Following this treatment, the patient's myeloma attained a first complete response, and he proceeded to autologous PBSCT. Pre-transplant evaluation included positive immunoglobulin G (IgG) antibodies against herpes simplex virus (8.64 IV) and HHV-6 (titer 1:320), consistent with latent infections, and negative IgG against cytomegalovirus and human immunodeficiency virus. Although the patient reported varicella infection in childhood, varicella-zoster IgG was negative. After collection of peripheral blood stem cells that were mobilized with granulocyte colony-stimulating factor (G-CSF) and plerixafor, the patient underwent autologous PBSCT following a preparative regimen of high-dose intravenous melphalan (200 mg/m^2); CD34+ cell dose was 10.94 x 10^6/kg. He received prophylactic acyclovir, ciprofloxacin, and fluconazole post-transplant.

On the first day post-transplant (day +1), he was febrile to 38.9°C and began empiric treatment with cefepime. Fever resolved, and bacterial cultures of blood and urine remained negative. On day +6, the patient had rigors and recurrence of fever to 40°C; at that time, he was

pancytopenic with a white blood cell (WBC) count of less than 0.1 x 10^9/L. Empiric vancomycin was added, and cefepime was continued. By day +7, fevers, rigors, and pancytopenia persisted, and the patient developed dyspnea, tachycardia, diarrhea, hypotension, and a diffuse erythematous macular and papular rash. Repeat bacterial cultures of blood and urine, a multiplex viral respiratory panel, and assay of stool for *Clostridium difficile* toxin were all negative. On day +9, the patient continued to have fever and rash and sustained a cardiac arrest, for which he required intubation and several days of pressor support with phenylephrine, vasopressin, and norepinephrine. On day +11, a skin biopsy showed both interface and spongiotic dermatitis with some eosinophils and extravasated red cells, without dyskeratotic cells or vasculitis. Therefore, skin biopsy was not consistent with typical findings of drug reaction or graft-versus-host disease. Peripheral blood studies at that time showed the WBC count was 11.2 x 10^9/L, of which approximately 60% were atypical lymphocytes (Figure 1); the absolute neutrophil count (ANC) was zero. Flow cytometry showed that these lymphocytes were positive for both CD4 and CD8 (Figure 2). Quantitative PCR for CMV and for Epstein-Barr virus DNA were negative. However, on day +11, a quantitative PCR for HHV-6 DNA was 250 copies/mL.

By day +14, both fever and rash had resolved. The abrupt and precipitous atypical lymphocytosis was then followed by prolonged pancytopenia (Figure 3). By day +30, the absence of WBC or ANC recovery was of concern for primary graft failure. A bone marrow aspirate and biopsy on day +31 showed 80-90% cellularity with marked erythroid hyperplasia (M:E ratio 0.18) and a small population (1.7%) of T-cells coexpressing CD4 and CD8. On day +34, the patient received a second autologous PBSC infusion (CD34+ cell dose, 10.94 x 10^6/kg). By day +38 (four days after the second PBSC infusion), the ANC was 0.99 x 10^6/L.

On day +34, the patient was extubated. He had several other comorbidities and complications including deconditioning, poor caloric intake, and intermittent atrial fibrillation. On day +54, he was discharged from the hospital. Evaluation at day +94 showed complete response of myeloma, with no monoclonal proteins, normal free light chain ratio, and negative serum and urine immunofixation.

DISCUSSION

We believe that this report is instructive because of the occurrence of both atypical lymphocytosis with CD4+/CD8+ cells and prolonged pancytopenia associated with HHV-6 reactivation after autologous HCT. In immunocompetent patients, infection with HHV-6 can present with mononucleosis-like manifestations, including absolute and atypical lymphocytosis[11,12]. However, these peripheral blood findings have not previously been observed in allogeneic or autologous HCT recipients who develop HHV-6 reactivation, whether during post-transplant pancytopenia or following hematopoietic engraftment recovery. The onset of HHV-6 viremia correlates with lymphocyte and monocyte recovery following allogeneic HCT, albeit at low levels of those cells (0.05×10^9/L)[18]; in contrast, we observed an ALC that was almost 100 times that level. The development of absolute and atypical lymphocytosis in our patient was concomitant with persistent fevers, rash, and positive quantitative HHV-6 DNA by PCR. The viral load in this patient was comparable to that reported in allogeneic HCT recipients with HHV-6 reactivation (median, 138 copies/mL)[10]. The immunophenotypic profile of the atypical lymphocytes showed coexpression of CD4 and CD8, a population of lymphocytes that is generally confined to the thymus during T-cell maturation. However, these cells can also be found sparingly in the peripheral circulation of healthy individuals[16]. Although the exact purpose

of these circulating double positive T-lymphocytes is unknown, it is believed that they play a role in fighting viral infections[16]. Additionally, HHV-6 has been reported to induce expression of CD4 in peripheral CD8+ lymphocytes through *de novo* expression of CD4 messenger RNA and subsequent glycoprotein[17], therefore, it is likely that viral reactivation led to atypical lymphocytosis with this characteristic immunophenotype.

Previous reports have indicated either no or minimal (1-3 day) delay in neutrophil engraftment after autologous PBSCT in patients with HHV-6 reactivation compared with those in whom viral reactivation did not occur[1,2,19]. In our case, neutrophil recovery did not occur until 38 days after first PBSCT. Extreme delay in neutrophil recovery after autologous PBSCT is decidedly uncommon; in 397 patients undergoing first autologous PBSCT, median time to neutrophil engraftment was 12 days (range, 9 to 25 days)[13]. Although the patient received a second infusion of autologous PBSCs at day +34 because of concern for primary graft failure, the recovery of neutrophils just four days later strongly indicates that the initial transplant was responsible for hematopoietic recovery. Additionally, our patient had prolonged thrombocytopenia; only at 47 days post-transplant did the platelet levels exceed 50 x 10^9/L without transfusion. In contrast, the median time to platelet engraftment after autologous PBSCT was 15 days (range, 10 to 27 days)[13].

Several *in vitro* studies have confirmed the inhibitory effects of HHV-6 on hematopoiesis. For example, exposure to HHV-6 prevented cytokine-induced maturation of marrow-derived macrophage progenitors by approximately 90%[20]. Another study showed that HHV-6 not only negatively affected proliferation but also inhibited differentiation of marrow stromal cells, pluripotent stem cells, erythroid precursors and granulocyte/macrophage precursors[21]. The myelosuppression is mediated by production of viral-associated factors that

interfere with the responsiveness of hematopoietic progenitors to growth factors, and not directly by active HHV-6 replication[20].

It is important to note that there are two distinct variations of HHV-6: HHV-6A and HHV-6B. These variants share about 88% sequence homology and are morphologically similar, yet their biological, epidemiological and clinical differences have led to their recognition as two distinct viruses by the International Committee for Taxonomy of Viruses in 2012[22,23,24]. Both HHV-6 variants target the CD46 receptor and preferentially replicate in CD4+ cells[25]. However, HHV-6A tends to more efficiently target the different types of cytotoxic effector cells compared to its more restricted variant, HHV-6B[17]. HHV-6A has also been specifically linked to the induction of CD4 expression in CD8+ T cells[17]. In vitro studies have suggested that the variants of HHV-6 may have differential effects on hematopoiesis, with more inhibitory effects observed with HHV-6B than with HHV-6A[26]. Unfortunately, we were not able to identify the specific HHV-6 variant that became reactivated in our patient.

The determinants of HHV-6 reactivation after autologous HCT are not clear. Our patient had received the proteasome inhibitor bortezomib, the use of which is associated with increased risks of reactivation of other latent herpesviruses, including herpes simplex and herpes zoster[27]. That previous bortezomib treatment may increase the risk of HHV-6 reactivation after autologous HCT for myeloma is supported by a recent study that showed almost a twofold greater incidence of post-transplant HHV-6 reactivation in patients who had received bortezomib plus dexamethasone when compared with those who received thalidomide plus dexamethasone (19.5% versus 9.5%, respectively)[7].

Current treatment recommendations for newly-diagnosed myeloma include bortezomib, usually combined with lenalidomide and dexamethasone[28]. Additionally, autologous HCT is a

preferred component of comprehensive therapy of multiple myeloma, as it provides longer progression-free survival than conventional treatment[29]. In view of these factors, the potential association of bortezomib with HHV-6 reactivation, and our and other reports, both the transplant infectious diseases specialist and the transplant physician may need to have a lower index of suspicion for HHV-6 reactivation when assessing myeloma patients who develop persistent fevers, rashes, atypical lymphocytosis, or prolonged marrow hypoplasia after autologous PBSCT.

CONCLUSION

In conclusion, it is believed that the reactivation of HHV-6 lead to an atypical lymphocytosis involving circulating CD4 and CD8 positive lymphocytes, followed by a prolonged pancytopenia in a 63-year-old man who underwent autologous PBSCT for multiple myeloma. Modest delayed bone marrow engraftment has been associated with HHV-6 reactivation in prior literature[2]. However, to our knowledge, atypical circulating lymphocytes expressing both CD4 and CD8 have not previously been associated with PBSCT. There is still much to learn about HHV-6 reactivation in transplant recipients, however, it is clear that physicians must be aware of its association with prolonged marrow hypoplasia after autologous PBSCT.

CLINICAL PRACTICE POINTS

- Reactivation of latent human herpesvirus-6 (HHV6) is often seen after allogeneic hematopoietic cell transplantation (HCT). HHV6 reactivation following HCT has been associated with a slight delay in hematopoietic recovery.

- HHV6 is known to induce expression of CD4 in CD8+ lymphocytes, and that viral reactivation likely led to the observed CD4+/CD8+ lymphocytosis. CD4+/CD8+ lymphocytes can also be found sparingly in the peripheral circulation of healthy individuals.
- This instructive case demonstrates that HHV6 reactivation after autologous PBSCT can be associated with atypical lymphocytosis of characteristic CD4+/CD8+ cells and subsequent prolonged pancytopenia.
- As autologous HCT remains a component of MM comprehensive therapy, transplant infectious diseases specialists and HCT physicians are encouraged to have a low index of suspicion for HHV6 reactivation when assessing MM patients who develop persistent fevers, rashes, atypical lymphocytosis or prolonged marrow hypoplasia after autologous HCT.

AUTHOR CONTRIBUTIONS

S.J.R. collected data and drafted the report. J.D. and D.F. collected data, drafted figures and critically revised the manuscript. A.M.Y. was involved with the data collection, critical revision and approval of the report.

FUNDING

This manuscript did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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FIGURE CAPTIONS

Figure 1. Photomicrograph of Wright-Giemsa stained peripheral blood smear obtained at day +11 post-transplant, showing large atypical lymphoid cells with moderately-abundant deeply basophilic cytoplasm, irregular nuclear contours and condensed nuclear chromatin (100x).

Figure 2. Flow cytometric analysis of peripheral blood at day +11 post-transplant, showing the lymphocytes (green) to represent 92% of total events. The majority of the lymphocytes are CD3+ T-cells with a subset showing coexpression of CD4 and CD8 (20% of all lymphocytes). The events (pink) represent a significant number of red blood cells and cellular debris.

Figure 3. Levels of white blood cells, absolute neutrophils, absolute lymphocytes, and absolute atypical lymphocytes following autologous PBSCT for multiple myeloma.

Figure 1

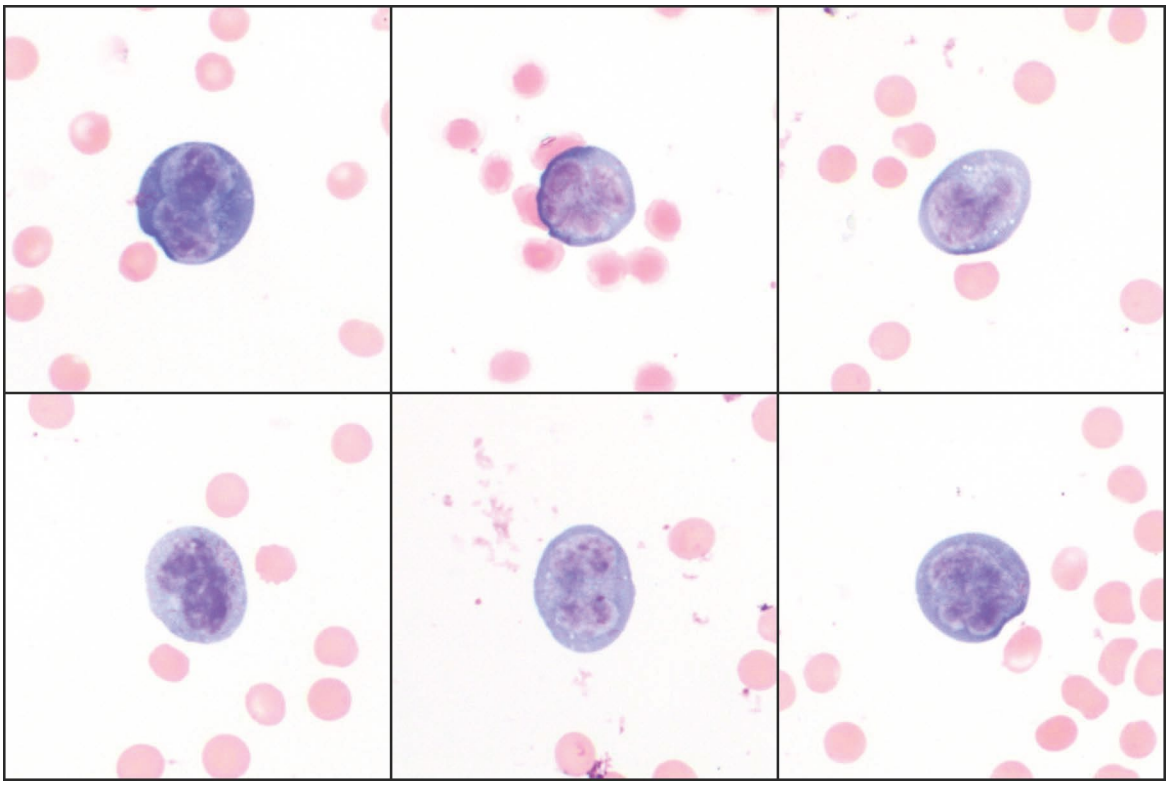

Figure 2

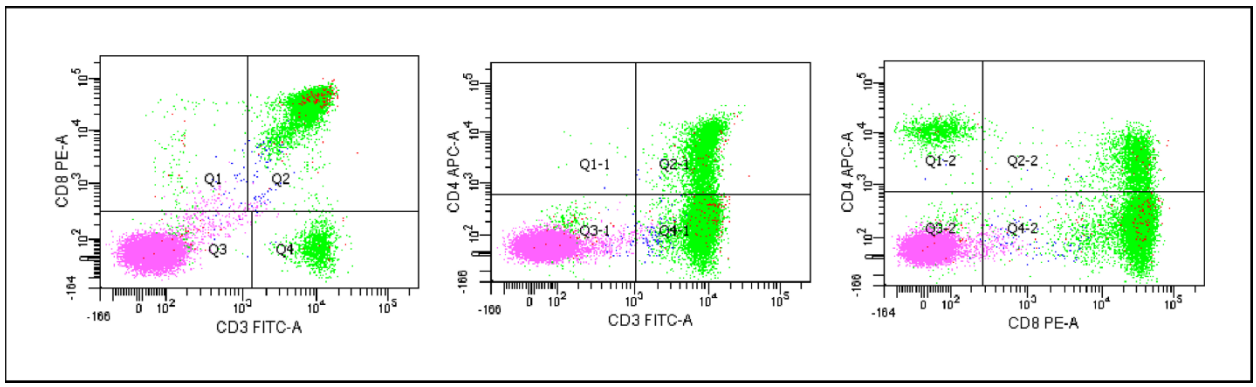
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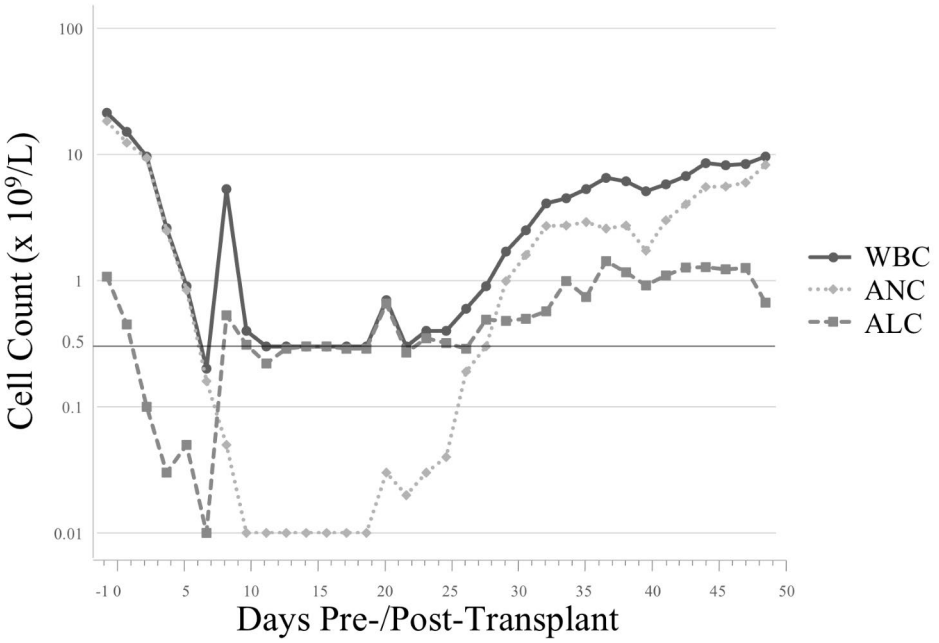
Figure 3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Days Pre-/Post-Transplant
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